

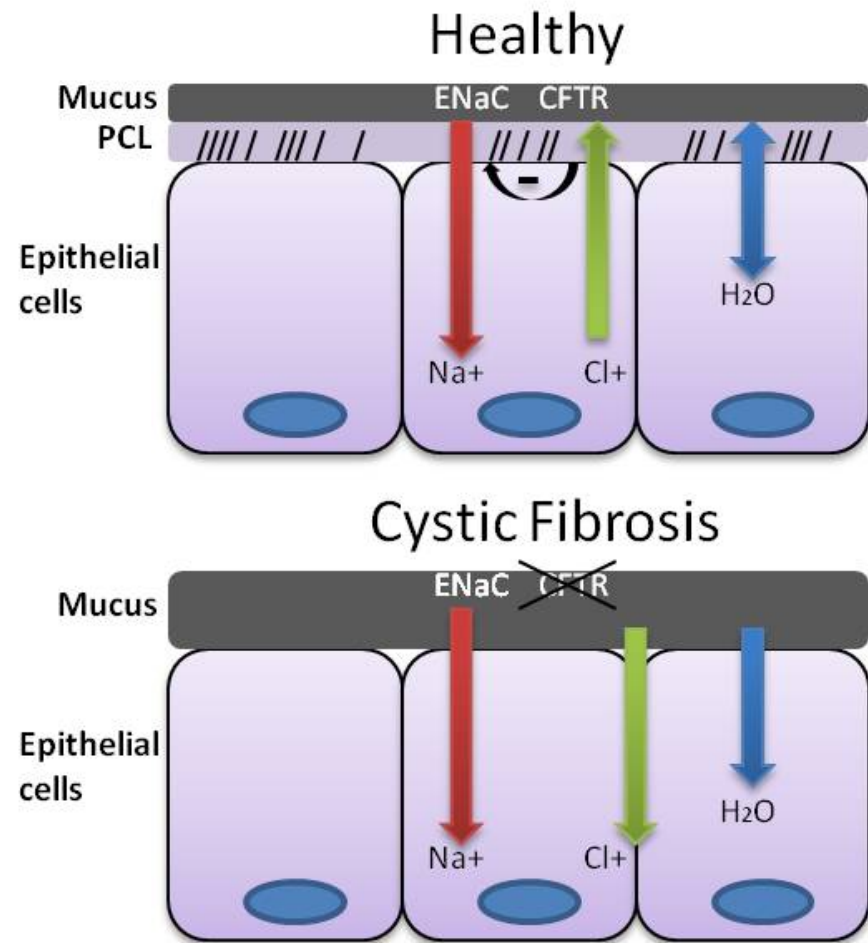
Characterization of *Pseudomonas aeruginosa* from cystic fibrosis lung infections

Introduction to diagnostic exercise

By Trine Markussen

Introduction

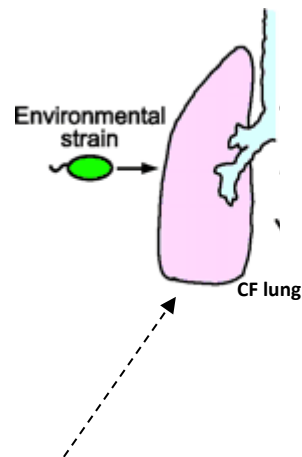
- Cystic fibrosis
- Autosomal recessive disorder
- Caused by mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene



Buchanan 2009

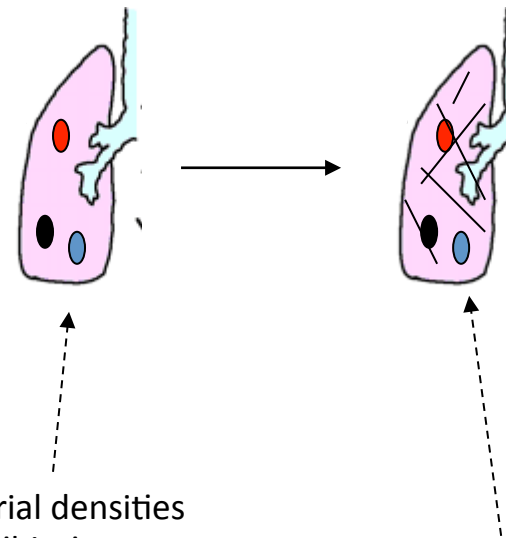
Model of *P. aeruginosa* infections in CF patients

Intermittent colonization (cycles of acute infections)



Periodic absence of *P. aeruginosa* in sputum, and no measurable antibodies in blood
Wild-type strains, relative susceptible to antibiotics

Chronic infection (decades)



High bacterial densities despite antibiotic treatment and actions of the immune system
Diversification

Airway inflammation and progressive lung damage
Selection

● → ●●● : Diversification: *P. aeruginosa* undergoes substantial genetic change during chronic infections

The Copenhagen *P. aeruginosa* strain collection

P. aeruginosa strains from CF patients have been collected since '73 by Niels Høiby

This unique strain collection represents more than 30 years of 'infection history' from a large number of patients

Infection dynamics in CF patients: A paleontological journey

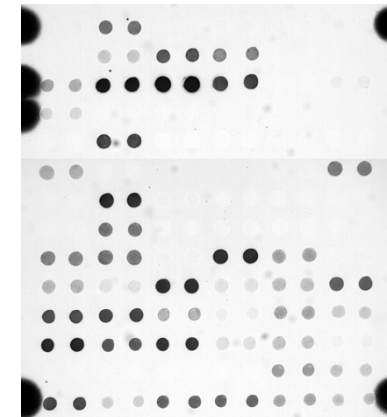
A paleontologist carefully chips rock from a column of dinosaur vertebrae



Genotyping of sequential isolates from different patients



A section of the 'frozen fossil collection' of bacteria isolated from CF patients



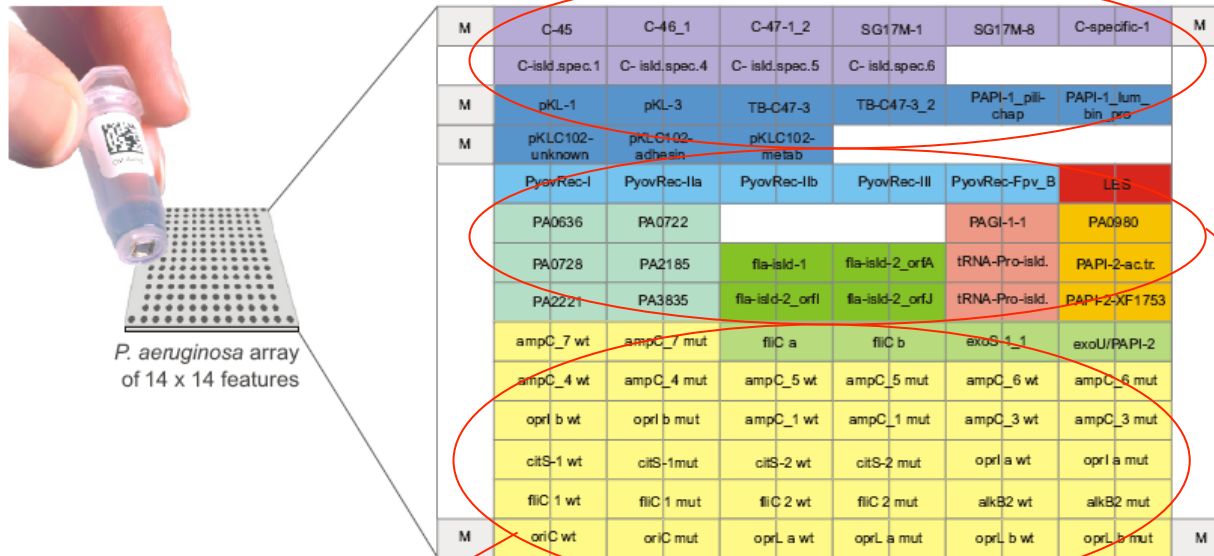
SNP based
genotyping

Paleontology is the study of the history of life based on fossil records

DNA-chip based genotyping of *P. aeruginosa*

Gene islands:

Fla- island (Lory), PAGI-1, -2 and -3, pKLC102, 47D7-island, PAPI-1, PAPI-2



P. aeruginosa array of 14 x 14 features

Outline of the Clondiag® chip

SNPs:

oriC, 2x *citS*, 6x *ampC*, *oprI*, *fliC*, *oprL*, *alkB2*

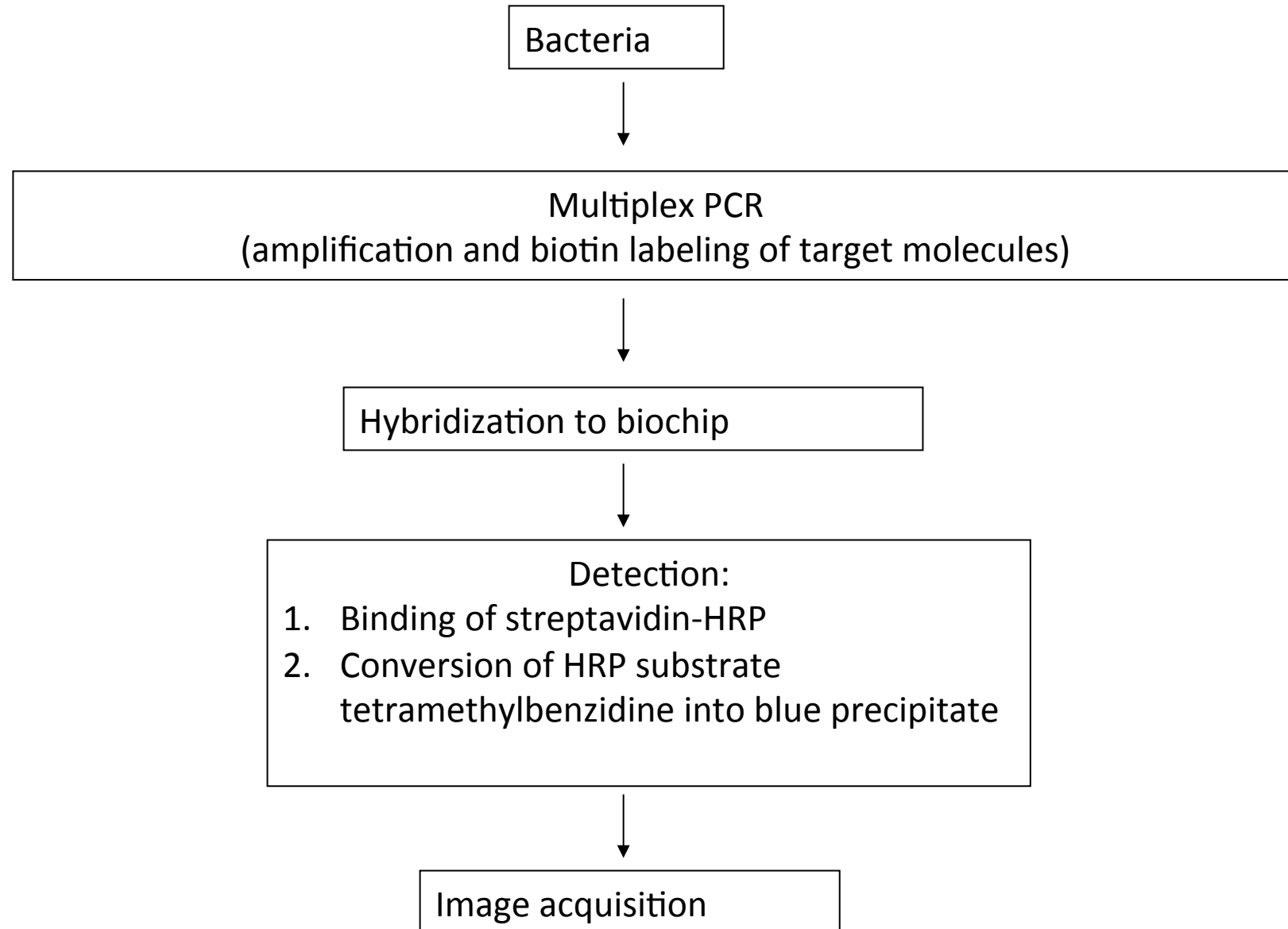
Variable genes & pathogenicity factors:

PA0636, PA0722, PA0728, PA0980, PA0987, PA2221, PA2387, PA3065, PA3291, PA3835, PA4101, *exoS*, *exoU*, *fliCa*, *fliCb*

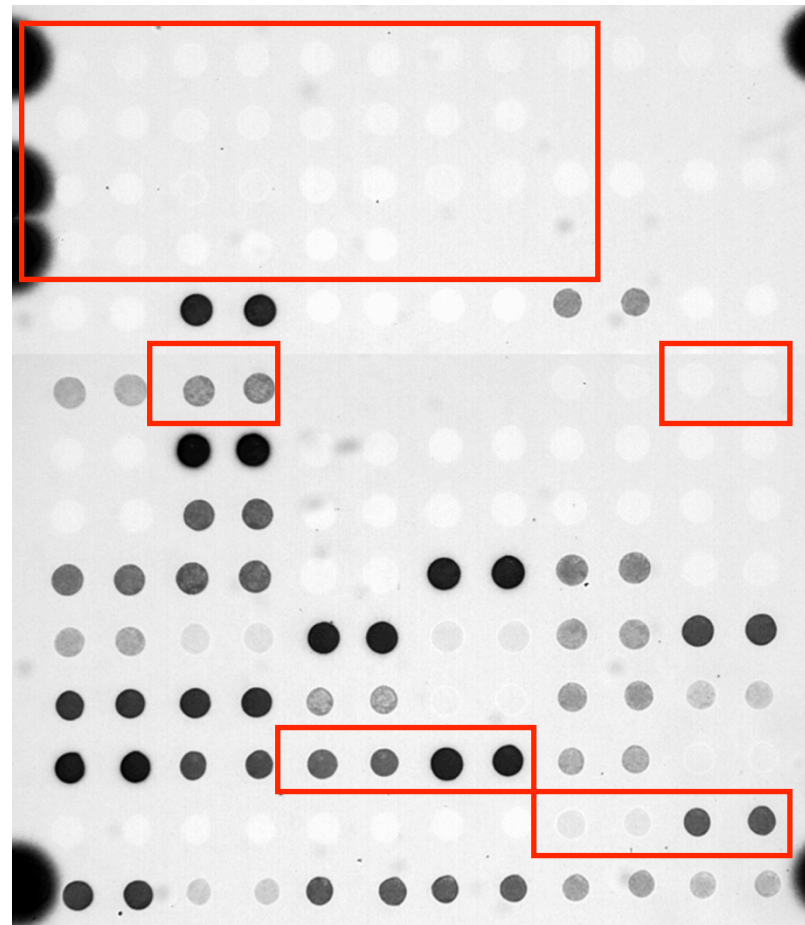
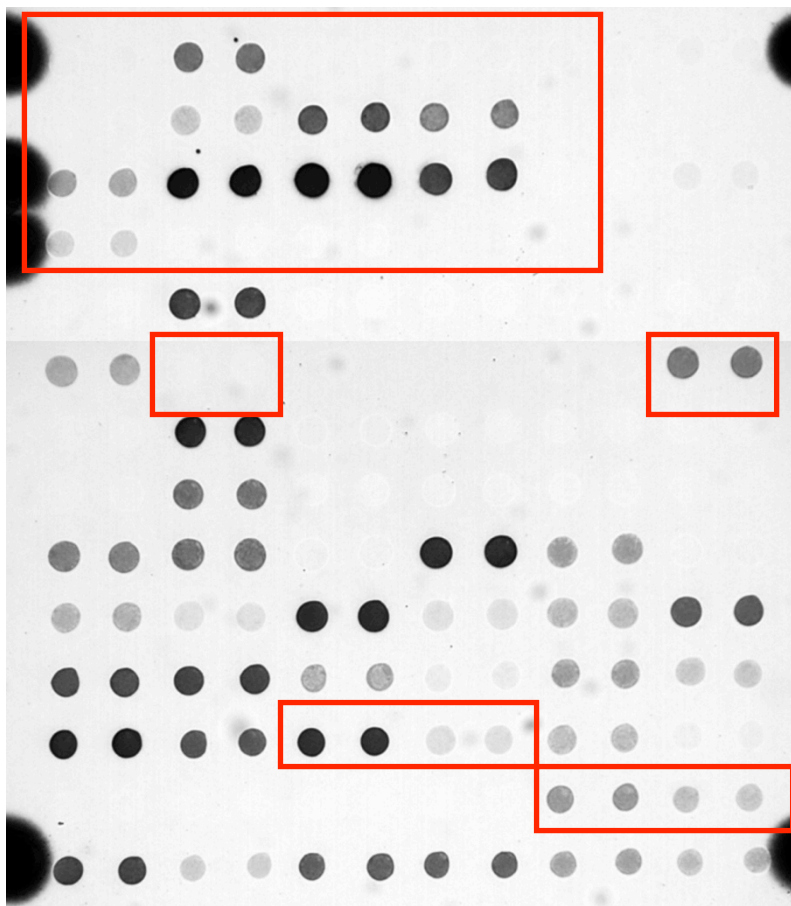
Unique sequences from SNPs (single nucleotide polymorphisms) of highly conserved genes.

Wiehlmann L et al (2007) PNAS 104(19):8101-6

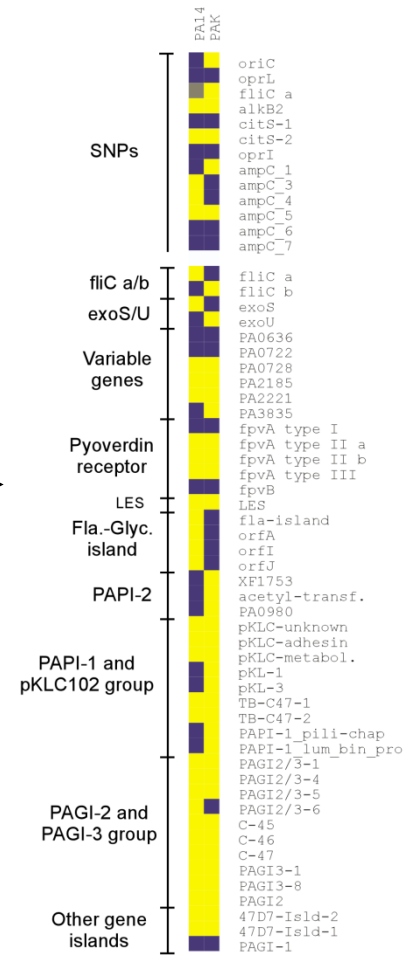
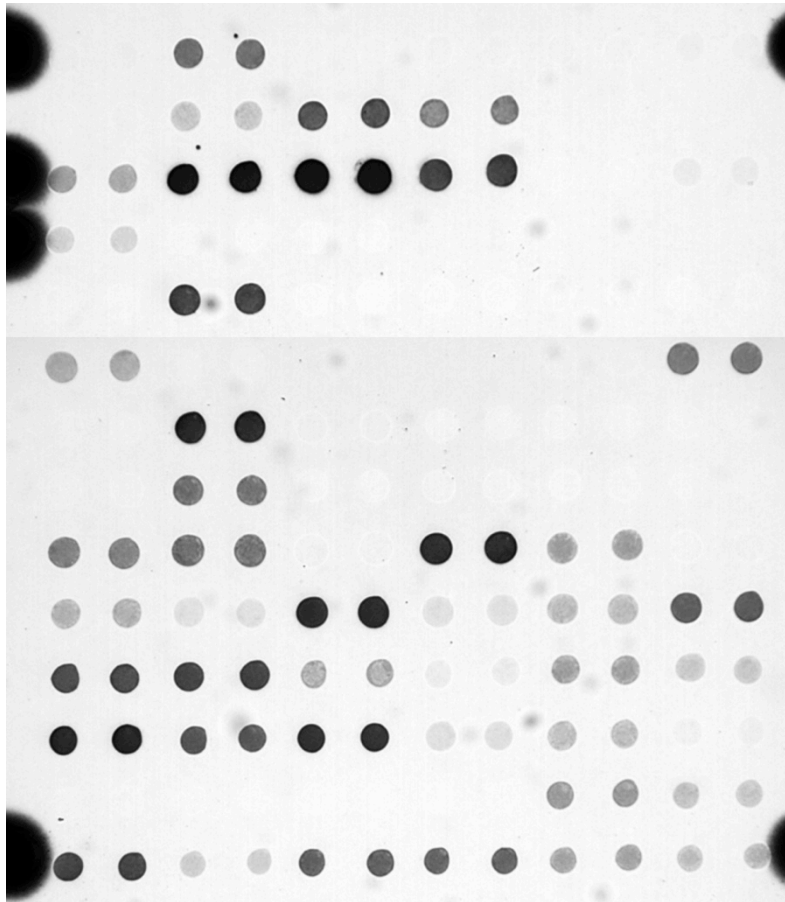
Protocol



Example: 2 different clones...



Data visualization



402A

'barcode'

'Data compression'
'Hexadecimal code'

Infection dynamics in long-term chronically infected CF patients

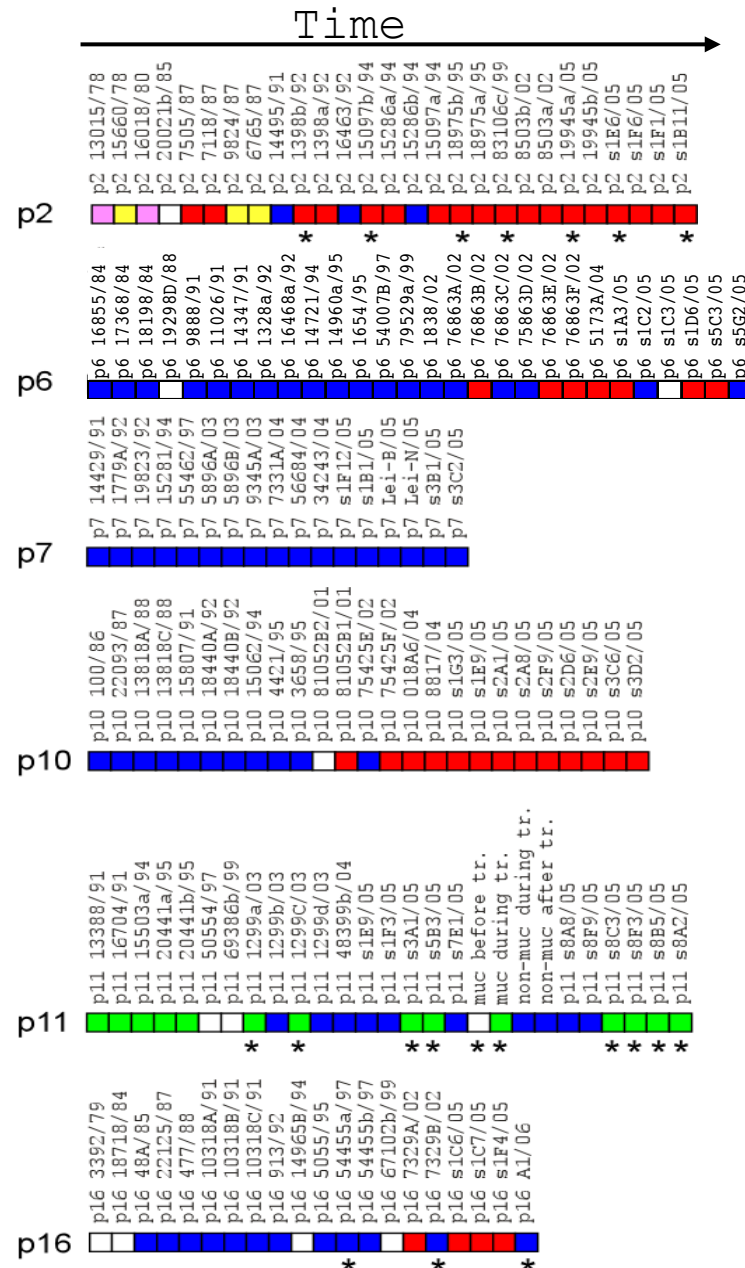
Genotype key:

Each **Color** indicate specific genotypes

No color indicate unique genotypes found only once

Phenotype key:

* = mucoid



Conclusions:

2 dominant clones: 'red' and 'blue'

6/6 random patients have become infected with one or both

Most long-term chronic infected patients carry 'red' and/or 'blue': We have identified the clones in >30 other patients.

The exercise

- 1 isolate
- Genotyping
 - AT-chip
- Phenotyping
 - Morphology
 - Antibiotic resistance
 - Virulence

Patient A

1	1992
2	1994
3	1994
4	1995
5	1995
6	1995
7	1995
8	1999
9	2002
10	2002

Introduction

Dictyostelium discoideum

- Complex eukaryote that lives part of its life as a unicellular amoeba
- Haploid – mutations are not masked by additional alleles.
- Feeds on bacteria

Pseudomonas aeruginosa

- Use similar virulence factors when infecting mammalian hosts or *Dictyostelium*
- Use 50% Standard Medium (SM)
- *Dictyostelium* can feed on *Pseudomonas*

Basic growth assay

PT5	WT PAO1 from Thila Koehler
PT531	PT5 $\Delta rhIR$ $\Delta lasR$
PT462	PT5 $\Delta rhIR$
PT498	PT5 $\Delta lasR$

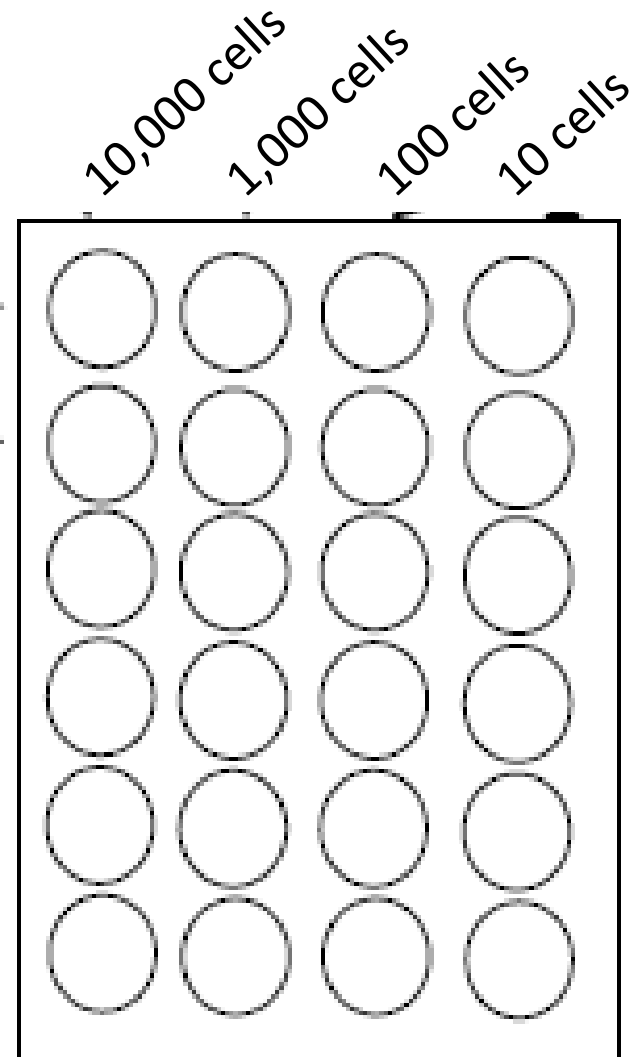
Isolate 1



Isolate 2



Isolate 3



Basic growth assay

Isolate	Day 4	Day 10	Score
PT5	0	0	0
PT531	4	4	8
PT462	2	4	6
PT498	0	0	0

